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【 CASE REPORT 】

Maximal Multistage Shuttle Run Test-induced Myalgia in a Patient with Muscle Phosphorylase B Kinase Deficiency

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Abstract:
Muscle phosphorylase b kinase (PHK) deficiency is a rare mild metabolic disorder caused by mutations of the *PHKA1* gene encoding the αM subunit of PHK. A 16-year-old boy experienced myalgia during the maximal multistage 20-m shuttle run test targeting the maximal oxygen consumption. Although an ischemic forearm exercise test was normal, a muscle biopsy revealed subsarcolemmal glycogen accumulation. He harbored a novel insertion mutation in the *PHKA1* gene that resulted in premature termination of the αM subunit close to the C-terminus. Compared with previously reported cases, his reduction in PHK activity was relatively mild.

Key words: muscle phosphorylase b kinase (PHK), αM subunit of the PHK gene (*PHKA1*), glycogen storage disease type IXd, maximal multistage 20-m shuttle run test

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Introduction

Phosphorylase b kinase (PHK) is a key enzyme involved in glycogen breakdown by phosphorylating glycogen phosphorylase, thereby inducing a change from its inactive (b) to active (a) form (1). PHK comprises four subunits with stoichiometry of $\alpha 4\beta 4\gamma 4\delta 4$. The γ subunit is catalytic, the α and β subunits are regulatory, and the δ subunit is identical to calmodulin. The α subunit has two different isoforms that show tissue-specific expression in the muscle (αM) or liver (αL) and are encoded by distinct genes on the X chromosome (*PHKA1* and *PHKA2*, respectively).

In previous studies involving enzyme activity measurements, muscle PHK deficiency presented with three clinical phenotypes (2): (i) juvenile and adult-onset exercise intolerance; (ii) late-onset slowly-progressive muscle weakness; and (iii) neonatal-onset floppy infant syndrome. To date, only eight cases of muscle PHK deficiency (glycogen storage disease type IXd) have been reported with the identification of mutations in the *PHKA1* gene encoding the muscle-specific αM subunit (1, 3-10).

We herein report a patient with muscle PHK deficiency caused by a novel mutation in the *PHKA1* gene and provide characteristic clinical pictures.

Case Report

A 16-year-old high-school boy was admitted to our hospital suffering from exercise intolerance in the form of myalgia. His birth and development were normal. He had enjoyed playing football, swimming, and gymnastics without exercise-induced myalgia until he was 15 years old, when his first episode of myalgia occurred during the maximal multistage 20-m shuttle run test (11) to target the maximal oxygen consumption in a handball club activity. Subsequently, he repeatedly suffered from muscle pain that gradually spread from the calf to the thigh during the incremental

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Table 1. Ischemic Forearm Exercise Test.

	Pre-exercise	Post-exercise (min)			
		0	2	4	6
Lactate (mg/dL)	14.1	50.7	37.1	29.9	25.5
Pyruvate (mg/dL)	0.53	1.00	0.77	0.76	0.73
Ketone (μmol/L)	54.5	31.2	41.4	45.1	45.7
Ammonia (μg/dL)	51	161	136	108	101

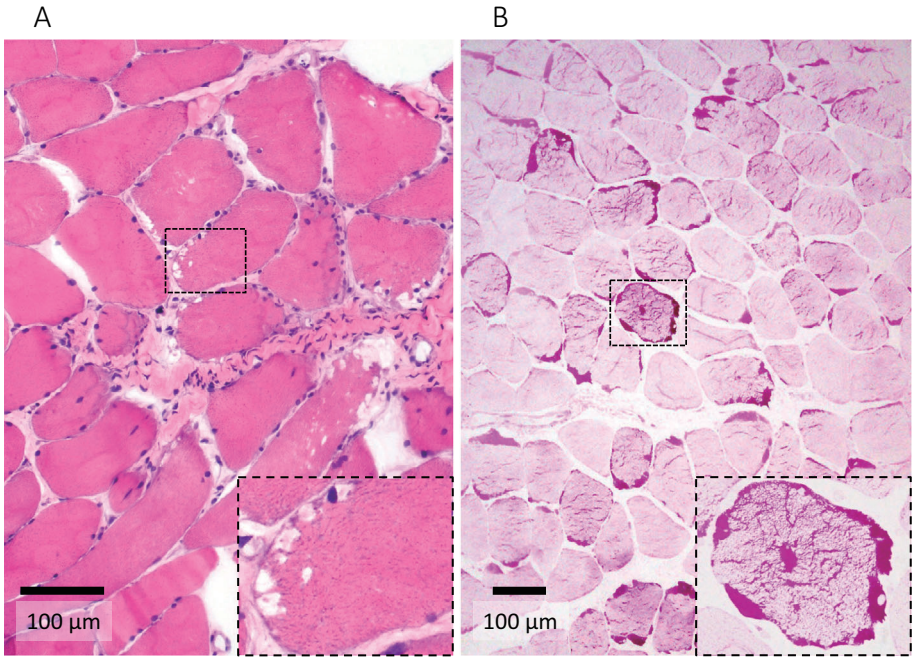


Figure 1. Fresh-frozen cryosections from the left vastus lateralis muscle. The subsarcolemmal vacuoles in some myofibers are filled with glycogen. (A) Hematoxylin and Eosin staining. (B) Periodic acid-Schiff staining of an epon-embedded section. Insets are enlarged images of the boxed regions.

exercise intensity associated with the shuttle run test. Occasionally, he had episodes of hyperventilation due to myalgia and noted pigmenturia after the muscle pain disappeared the next morning.

His parents were non-consanguineous. No other family members had similar symptoms. We decided to make a differential diagnosis of various diseases presenting with exercise intolerance and/or myalgia such as glycogen storage diseases, disorders of fatty acid metabolism, and mitochondrial myopathies.

Findings on a physical examination at admission were normal, with no muscle atrophy or weakness and no organomegaly. His serum creatine kinase (CK) levels were ranging from 213 to 2,027 U/L (normal range: 54-248 U/L). An ischemic forearm exercise test showed normal elevation (>5-fold) of plasma lactate (Table 1). However, a muscle biopsy from the left vastus lateralis muscle revealed subsarcolemmal glycogen accumulation in some myofibers (Fig. 1). Biochemical analyses demonstrated a decrease in PHK activity (4.5 nmol/min/mg protein; normal range: 18.6-68.6 nmol/min/mg protein; Table 2) in the muscle biopsy homogenate, but not in red blood cells (data not shown). Interestingly, the *in vitro* anaerobic lactate production rate was significantly decreased with glycogen as a substrate, but not glucose-1-P as a substrate, when the muscle biopsy homogenate was used as an enzyme source (Fig. 2).

Using a next-generation sequencer (IonPGM™; Thermo Fisher Scientific, Waltham, United States), targeted resequencing of 41 metabolic myopathy-related genes (12) identified a previously unreported mutation in the *PHKA1* gene, comprising a single nucleotide insertion that created a premature stop codon (c.3579_3580insT, p.S1194*) in exon 32 (Fig. 3). This mutation has not been reported in the public databases of the general population such as gnomAD, 1000 Genomes, ToMMo, and HGMD.

Discussion

Muscle PHK deficiency is a very rare disease among glycogen storage diseases. Table 3 summarizes the characteristics of nine cases of glycogen storage disease type IXd harboring mutations in the *PHKA1* gene, including our case. Of these, six cases showed exercise intolerance in the form of myalgia, cramps, or early fatigue; four exhibited progressive

Table 2. Activities of Glycolytic/glycogenolytic Enzymes in Skeletal Muscle.

Enzymes	Patient	Control Range
Phosphorylase (+AMP)	83.3	33.3-89.3
Phosphorylase b kinase	4.5	18.5-68.6
Phosphoglucomutase	267.7	221.1-562.0
Phosphohexoisomerase	1,231.9	669.1-1,204.0
Phosphofructokinase	135.2	33.7-88.5
Aldolase	323.1	246.7-580.0
Glyceraldehyde-3-P-dehydrogenase	1,643.8	1,577.8-3,107.2
Phosphoglycerate kinase	1,378.7	658.3-1,470.2
Phosphoglycerate mutase	830.2	687.7-1,491.5
Enolase	430.4	224.6-692.5
Pyruvate kinase	1,531.3	837.8-2,075.3
Lactate dehydrogenase	1,198.8	1,029.3-2,734.4

All enzymatic activities are expressed as nmol substrate utilized/min/mg protein.

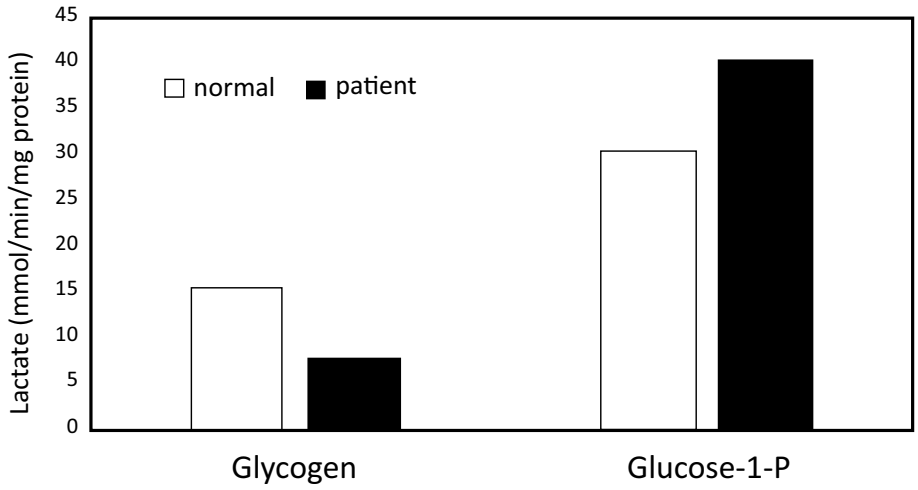
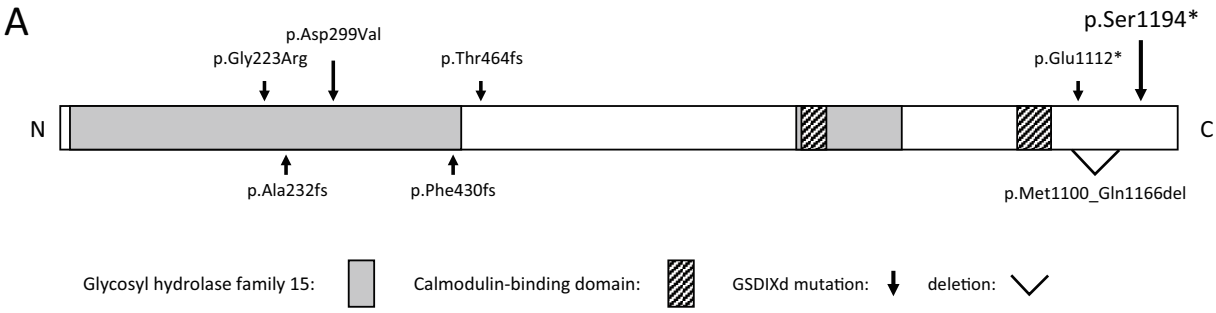


Figure 2. ***In vitro*** **anaerobic lactate production rates. Bars indicate the average values of duplicate measurements. The lactate production rate in the muscle biopsy homogenate was measured using glycogen or glucose-1-P as the substrate. Note that the breakdown activity of glycogen into glucose-1-P was impaired in patient.**

muscle weakness; and two had asymptomatic hyperCKemia. Since myophosphorylase activation is impaired under conditions of PHK deficiency, it is expected that symptoms similar to McArdle disease, which is caused by a primary myophosphorylase defect, may occur, such as exercise intolerance. However, in reality, patients with PHK deficiency present with extremely mild symptoms, sometimes being almost asymptomatic despite mild impairment of muscle glycogenolysis, elevated CK levels, and glycogen accumulation in the muscle. In the present case report, the patient had been asymptomatic during regular exercise until myalgia was first induced by the maximal multistage 20-m shuttle run test, a very-high-intensity exercise designed to target the maximal oxygen consumption (11). If he had not undergone the 20-m shuttle run test, he might not have been diagnosed with PHK deficiency.

Another pitfall in the diagnosis of PHK deficiency is that, unlike McArdle disease, lactate increases normally in a forearm ischemic exercise test, which is commonly performed as a screening test for glycogenosis. Interestingly, *in vitro* experiments revealed decreased lactate production when glycogen was supplied to the muscle biopsy homogenate from the patient despite a normal elevation of lactate in the forearm ischemic exercise test. This can be explained by the integration of various stimuli in the regulation of myophosphorylase activity *in vivo*. At the beginning of exercise, calcium stores are released from the sarcoplasmic reticulum, causing activation of phosphorylase kinase and subsequent phosphorylation and activation of myophosphorylase (13). Glycogen phosphorylase is allosterically activated by increased [Pi] within the cell and is activated by high AMP levels (1). The ischemic forearm exercise test is not diagnostically helpful in patients with PHK deficiency, as these variable factors co-regulate glycolysis.

Our patient harbored a novel mutation in the *PHKA1* gene that resulted in premature termination of the αM



B

Ser1194* S-farnesyl cysteine site

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Homo sapiens            1167 KTLGADDTMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSICAMQ 1223
Macaca mulatta          1167 KTLGADDIMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSICAMQ 1223
Pan troglodytes         1133 KTLGADDTMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSICAMQ 1189
Mus musculus            1185 KTLGADDTMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSLCAMQ 1241
Rattus norvegicus       1186 KTLGADDIMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSLCAVQ 1242
Canis lupus familiaris  1183 KTLGADDTMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSICAMQ 1239
Bos taurus              1170 KTLGADDIMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSICAMQ 1226
Oryctolagus cuniculus   1181 KTLGADDIMLAKDPASGICTLLYDSAPSGRFGTMTYLSKAAATYVQEFLPHSICAMQ 1237
Gallus gallus           1181 KALGADDQMLEKDPSTGICDLLYDSAPSGRFGTMTYLSKSVALHLYDLLPGDGCAMQ 1237
Xenopus tropicalis      1135 KVLGADDTMLDVDPTTGICHLLYDSAPSGRFGTMTYLSKSVAITLQESLPHSGCSMQ 1191
Danio rerio             1176 KDLGAEDSILEKDQSTGICRLLYDTAPSGRFGTMTYLTKAVATYAQDFLPSGACTVQ 1232
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Figure 3. Mutations found in the *PHKA1* gene. (A) Structure of the αM subunit of PHK and sites of mutations of the *PHKA1* gene reported in patients with muscle PHK deficiency. (B) Amino acid alignment of the C-terminus of the αM subunit of PHK and a novel mutation for muscle PHK deficiency. A premature stop codon in Ser1194 leads to a defect in the S-farnesyl cysteine site. GSD IXd: glycogen storage disease type IXd

Table 3. Patients with Glycogen Storage Disease Type IXd Harboring Mutations in the *PHKA1* Gene.

	Mutations	Sex/age (yr)	Age at onset	Chief complaint	Exercise intolerance			Muscle weakness	Hyper-CK-emia	Increased lactate in ischemic forearm test	Muscle PHK activity (% of lower control value)	References
					Myalgia	Cramps	Early fatigue					
1	c.3334G>T (p.Glu1112*)	Male/64	46 yr	Gait disturbance	–	–	–	+	+	–	0.5	4, 6
2	c.3498+1G>C (p.Met1100_Gln1166del)	Male/28	15 yr	Exercise intolerance	+	–	+	+	+	–	Undetectable	5, 7
3	c.896A>T (p.Asp299Val)	Male/18	6 yr	Exercise intolerance	+	+	+	+	+	+	8.9	3, 4
4	c.695del (p.Ala232fs)	Male/56	6 mo	Myalgia	+	–	+	+	+	ND	Undetectable	8
5	c.667G>A (p.Gly223Arg)	Male/50	Childhood	Exercise intolerance	–	+	+	–	+	+	29	1
6	c.1394del (p.Thr464fs)	Male/17	17 yr	HyperCKemia (asymptomatic)	–	–	–	–	+	ND	6.4	9
7	c.1293del (p.Phe430fs)	Male/39	32 yr	Myalgia	+	–	–	–	+	+	16.8	10
8	c.695del (p.Ala232fs)	Male/69	64 yr	HyperCKemia (asymptomatic)	–	–	–	–	+	+	22.4	10
9	c.3579_3580insT (p.Ser1194*)	Male/16	15 yr	Myalgia	+	–	+	–	+	+	24	Present case

ND: not done

subunit of PHK close to its C-terminus (Fig. 3A). This mutation is considered to cause a defect in the S-farnesyl cysteine site (Fig. 3B), which plays a role in directing the αM subunit to the membrane (14, 15). Although the decrease in enzyme activity was relatively mild in the present case, no correlations between the mutation site, residual muscle PHK activity, or severity of clinical symptoms were found in comparison with previously reported cases (Table 3). Unfor-

tunately, genetic testing of family members was not allowed, so we were unable to conclude whether the patient's genetic mutation occurred in *de novo* or was due to X-linked inheritance.

In conclusion, a submaximal to maximal incremental aerobic exercise test can facilitate the diagnosis of muscle PHK deficiency (glycogen storage disease type IXd), which may be underdiagnosed because of the very mild symptoms and normal reaction in the ischemic forearm exercise test. It is important to include this disease in the differential diagnosis of exercise intolerance.

The authors state that they have no Conflict of Interest (COI).

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